GONORRHOEA

Finding, confirming, and managing gonorrhoea in a population screened for chlamydia using the Gen-Probe Aptima Combo2 assay

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Objectives: To identify the prevalence of *Neisseria gonorrhoeae* (NG) within a population screened for *Chlamydia trachomatis* (CT). To monitor confirmatory microscopy, culture, and partner findings following reactive Aptima Combo2 assay (AC2) gonorrhoea screening tests.

Methods: Between June and December 2004, all gonorrhoea screening tests performed using AC2 for clients taking part in the Liverpool Chlamydia Screening Programme were monitored. Clients with AC2 NG reactive results were referred to a local genitourinary medicine (GUM) department for confirmatory microscopy, culture, treatment, and partner follow up.

Results: 47 (1%) of 4680 women and eight (1.7%) of 473 men had AC2 reactive gonorrhoea screening tests. Of those clients who agreed to follow up and were tested before any treatment, supportive evidence for a gonorrhoea diagnosis was found in 37 (97%) of 38 women and all five men. In the population opportunistically screened for chlamydia, CT prevalence rates were 12% for women and 15.7% for men. Although both women and men showed a higher relative risk for NG if chlamydia positive, of the 47 women who were reactive for NG by AC2, 55% (26) were negative for chlamydia.

Conclusions: Sexually transmitted infections are rising in England and reduction of gonorrhoea rates is an objective of the Department of Health Sexual Health and HIV Strategy. AC2 tests provide an acceptable and accurate means of testing for gonorrhoea in an asymptomatic population in the community. AC2 had a higher positive predictive value than might be suggested by previous clinical trials in this low prevalence population. Although antibiotic sensitivity must be monitored, AC2 testing may offer a more acceptable alternative to microscopy and culture for NG in some populations.

Infection by the organism *Neisseria gonorrhoeae* (NG) is the second most common bacterial sexually transmitted infection (STI) in England and Wales. The number of diagnoses of gonorrhoea has risen by 137% between 1995 and 2003. The North West Region saw 2886 new diagnoses in 2003.[1]

The Department of Health's 2001 Sexual Health and HIV Strategy identified the reduction by 25% of newly acquired gonorrhoea infections by 2007 as one of its objectives.[2] Another key feature of the strategy was to introduce a national chlamydia screening programme (NCSP), following successful pilot studies that began in 2000. The NCSP aims to provide opportunistic screening and treatment of genital *Chlamydia trachomatis* (CT) infection for asymptomatic men and women aged under 25 years. At present, the NCSP covers 30% of all sexually active people aged 15–24 years in England.[3]

The Liverpool and South Sefton Chlamydia Screening Programme (LSSCSP) is part of phase 2 of the roll out. Screening is undertaken using Gen-Probe Aptima Combo-2 (AC2), a nucleic acid amplification test (NAAT). Screening gradually started in contraceptive clinics, GP surgeries, NHS walk-in centres, abortion providers, and prisons. Before the introduction of screening, a local steering group comprised key members of the screening sites and LSSCSP staff agreed that concomitant gonorrhoea screening would be offered. This decision was as a result of the introduction of the new technology, AC2, which allowed testing for chlamydia and gonorrhoea using the same specimen. This enabled the programme to identify cases of gonorrhoea in a non-genitourinary medicine (GUM) setting as well as assessing the risk of gonorrhoea in an asymptomatic population without incurring any additional cost per test. It was decided from the outset that the results of AC2 testing for NG would be monitored to assess prevalence and false positive results, which have been known to occur with other NAATs for NG.[4]

METHODS

From 9 June 2004, screening for chlamydia and gonorrhoea was offered to all men and women aged under 25 years of age accessing locations participating in the LSSCSP.

Clients were provided with written information about the NCSP and chlamydia and gonorrhoea screening before their consultation with a health professional. Clients were advised, before testing, that treatment for gonorrhoea was not available within the NCSP but that they would be advised how to access treatment in the event of a positive result. For this reason, separate consent was sought for gonorrhoea screening, with the health professional being required to tick an additional section of the screening request form.

Specimens used were: men, first void urine (FVU) after at least 1 hour bladder retention; women, endocervical swab if already being examined *or* FVU after at least 1 hour bladder retention *or* self taken vulvovaginal swab.

Three proprietary specimen collection kits provided with the AC2 allowed endocervical swabs, vulvovaginal swabs,

Abbreviations: AC2, Aptima Combo2 assay; CSO, chlamydia screening office; CT, *Chlamydia trachomatis*; FVU, first void urine; GRASP, The Gonococcal Resistance to Antimicrobials Surveillance Programme; GUM, genitourinary medicine; LSSCSP, Liverpool and South Sefton Chlamydia Screening Programme; NAAT, nucleic acid amplification test; NCSP, National Chlamydia Screening Programme; NG, *Neisseria gonorrhoeae*; STI, sexually transmitted infections

Table 1 Detection rates for CT and GC by gender and reason for testing

	NG positive (%)	NG negative	Total
Men			
Screening:			
CT positive	6 (11.5%)	46	52
CT equivocal	0	3	3
CT negative	2 (0.73%)	274	276
Contacts of CT:			
CT positive	0	87	87
CT equivocal	0	1	1
CT negative	0	54	54
Women			
Screening:			
CT positive	20 (3.6%)	538	558
CT equivocal	1 (2%)	50	51
CT negative	26 (0.65%)	4007	4033
Contacts of CT:			
CT positive	0	18	18
CT equivocal	0	3	3
CT negative	0	17	17

and stabilised urine to be transported to the laboratory at ambient temperature.

Laboratory tests for CT and NG were conducted using AC2 according to the manufacturer's instructions (GenProbe, San Diego, CA, USA). Briefly, AC2 uses magnetic target capture, transcription mediated amplification, and dual kinetic (fluorescence) assay to detect RNAs of both CT and NG within the same reaction tube. In accordance with protocol for the NCSP, all initially reactive AC2 tests were repeated on the residual sample to provide confirmation. This algorithm for repeat testing as confirmation is currently issued in England as a suggested national standard operating procedure[5]

The test results were sent to a central chlamydia screening office (CSO) and the client contacted by the method they had previously agreed on accessing screening. On receipt of an AC2 reactive NG result, the client was informed that a diagnosis of gonorrhoea had been suggested and that further microscopy and culture were necessary to confirm the diagnosis and test antibiotic sensitivity. Arrangements were then made to ''fast-track'' the client to a GUM clinic either within the local hospital or an outreach GUM service within a contraceptive clinic.

Clients who consented to confirmatory testing were examined for NG by conventional microscopy and culture from the urethra, cervix, rectum, and pharynx, as appropriate. Antibiotic treatment for NG, and CT if positive, was given.

Gram stains were read by GUM based biomedical scientists (who for internal quality control re-examine negative slides from culture positive patients).

Culture for NG was at the bedside via swabs from female cervix, urethra, rectum, and pharynx and for males, endourethral loops and swabs for rectum and pharynx. NG selective medium was used (BioConnections, Wetherby, Yorks, UK). In-house growth supplements of L-glutamine, ferric nitrate, and co-carboxylase were added, plus antimicrobial agents; vancomycin, colistin, ampohtericin, and trimethoprim. On NG positive microscopy, an additional NG selective medium was used containing growth supplements as above, but with alternative antimicrobial selective supplement (LCAT, BioConnections, Wetherby, Yorks, UK) replacing vancomycin with lincomycin. Plates were incubated in carbon dioxide (7%) at 37℃ for 48 hours. Identification was by Phadebact (Boule diagnostics AB, Sweden).

NG culture and antibiotic sensitivity were the basis for any additional action required. Partner notification was initiated by a health adviser within the GUM setting in accordance with national guidelines produced by the Society of Sexual Health Advisers in 2004,[6] and outcomes reported back to the CSO.

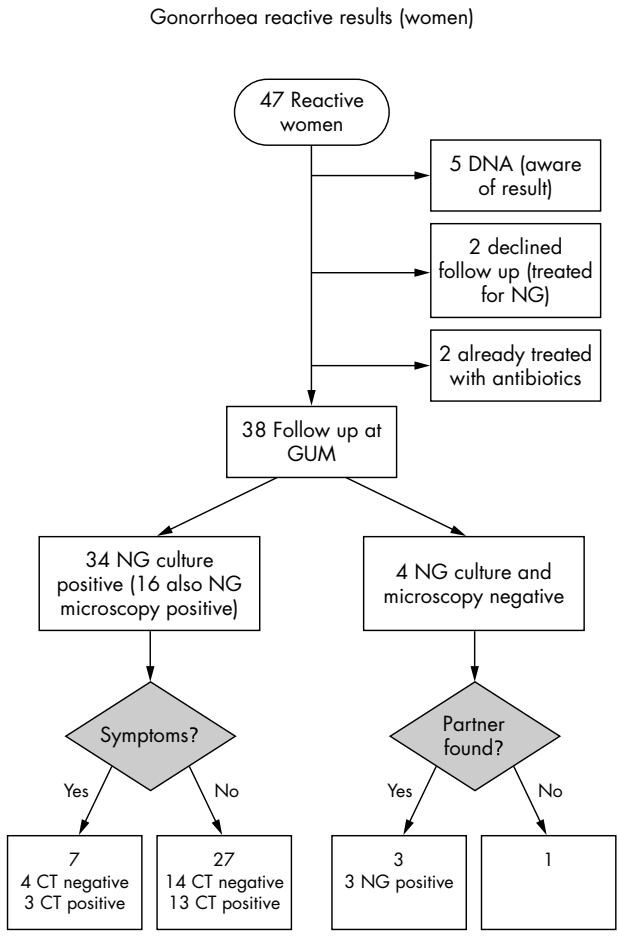

Figure 1 Follow up of AC2 NG reactive results (women).

Statcalc in EpiInfo version 6 was used to assess relative risk with Taylor series 95% confidence limits, and p values from χ^2 with Yates's correction or from Fisher's exact test.

RESULTS

Between 9 June and 31 December 2004, 5427 clients (491 men and 4936 women) accessing screening sites within the LSSCSP were tested for CT. Of these, 5153 (95%) agreed to be screened for NG (473 men and 4680 women).

Detection of chlamydia and gonorrhoea

Table 1 shows the detection rates for CT and NG by gender and reason for testing.

The overall positivity rate of chlamydia was 13.9% (715/5153). Of these 715, 105 cases were known to be contacts of chlamydia positive partners. Hence, the prevalence (the rate among the clients who were screened opportunistically) was 12.3% (610/4973). The positivity rate in women was 12.3% (576/4680) while the prevalence was 12% (558/4642). For men, the positivity rate was 29.4% (139/473) and prevalence 15.7% (52/331).

The positivity rate of AC2 reactive NG results was 1.07% (55). The positivity rate for women was 1% (47) and for men 1.7% (8). Both men (RR 16.1, CI 3.34 to 77.6) and women (RR 5.42 CI 3.06 to 9.6) showed a higher relative risk for NG if positive for CT.

Confirmatory testing

Of the 47 women whose AC2 results were reactive for NG (fig 1), nine did not undergo confirmatory testing. Four were seen for follow up but refused further testing although they accepted antibiotics. The other five were fully aware of their result but chose not to attend any follow up. Of the 38 women who underwent confirmatory testing, 34 (89%) were proved positive on microscopy and/or culture. Less than half of those with positive culture had positive microscopy.

Thirty four women gave a positive culture for gonorrhoea and a further three were contacts of men with confirmed gonorrhoea. Hence, supportive evidence of gonorrhoea was found in 37 of 38 women. The remaining woman was seen for confirmatory testing more than 3 months after the original AC2 result and her partner was not tested.

Eight men had AC2 results reactive for NG. One was aware of his result but chose not to attend any follow up and two others refused further testing but accepted antibiotics. Of the five men who underwent confirmatory testing, all were confirmed by microscopy and culture

Sites of infection

In women AC2 urine tests showed a slightly higher positivity rate for NG than AC2 swabs. This may be stratified as 1.06% for urine (40 out of 3786 tests) 0.85% for vulvovaginal swabs (5/589 tests), and 0.66% for cervical swabs (2/305 tests). However, the difference between urine and swabs was not statistically significant ($p = 0.38$).

Microscopy and culture was undertaken from urethral swabs for men, and urethral, cervical and, in some cases, rectal swabs for women. Pharyngeal swabs were also taken for culture where indicated by the history. Culture confirmed positive microscopy results in all instances. However, for women, only 13/26 (50%) positive cervical culture results where initially seen on microscopy. In addition, only 9/22 (41%) positive urethral cultures and 3/13 (23%) positive rectal cultures were found on microscopy.

Results were positive at multiple sites for 68% of women and 20% of men. Only the cervical swab was positive for 23.5% of women and only the urethral swab in a further two women (6%). One woman (3%) was positive on pharyngeal swab only. The patterns of NG positivity by culture from various sites are set against the sample reactive by AC2 in table 2. Culture from the cervix, which is often taken as the gold standard, was not found to be positive in several cases.

Symptoms

The NCSP is aimed at asymptomatic clients only and ideally those with symptoms should be seen at a GUM clinic. However, often, the symptoms are minor or the clients are not willing to address the issue at the time. Therefore in the absence of fully integrated services, a pragmatic approach was adopted. Case notes of clients with gonorrhoea AC2 reactive results were reviewed to see if any symptoms had been documented at the time of screening. Forty women (85%) and five men (62.5%) were asymptomatic. Of the three men and seven women who reported symptoms, all were confirmed positive for gonorrhoea on culture. Concomitant CT infection was found in two symptomatic men (66%) and three symptomatic women (43%) (fig 1).

Symptoms reported in men were penile discharge (3/3) and in women, vaginal discharge (4/7), irregular vaginal bleeding (3/7), and back and abdominal pain (1/7).

Table 2 Pattern of positive culture results compared with AC2 test (women)

Original positive AC2 test	No	NG culture results			
		Cervix	Urethra	Rectum	Pharynx
Cervical swab	1	+	−	−	−
Cervical swab	1	−	+	+	−
Urine	1	+	+	+	+
Urine	5	+	+	+	−
Urine	1	+	+	+	n/t
Urine	5	+	+	−	−
Urine	1	+	+	−	n/t
Urine	1	+	+	n/t	n/t
Urine	1	+	−	+	−
Urine	5	+	−	−	−
Urine	2	+	−	n/t	−
Urine	1	+	−	n/t	n/t
Urine	2	−	+	+	−
Urine	1	−	+	+	n/t
Urine	1	−	+	−	−
Urine	3	−	−	−	−
Urine	1	−	−	n/t	−
Vulvovaginal swab	1	+	+	+	−
Vulvovaginal swab	1	+	−	−	−
Vulvovaginal swab	1	−	+	−	+
Vulvovaginal swab	1	−	+	−	−
Vulvovaginal swab	1	−	−	−	+

+, positive culture result; −, negative culture result; n/t, site not tested.

Key messages

- Concomitant screening for gonorrhoea within a chlamydia screening programme is acceptable to clients
- A number of clients with asymptomatic gonorrhoea would not have been diagnosed if concomitant chlamydia and gonorrhoea screening was not undertaken
- Screening for gonorrhoea, using AC2 testing, is a reliable indicator of the presence of gonorrhoea infection

DISCUSSION

Concomitant gonorrhoea screening within a population screened for chlamydia has resulted in the diagnosis and treatment of largely asymptomatic gonorrhoea infection found within primary care settings. Previously the policy in the one young person's clinic where CT screening was available, was that only those clients who had a positive CT result were considered for NG testing.[7] If the same principle had been applied here, 28 (51% of total; 55% in women; 25% in men) of the AC2 NG positive results would not have been found.

The use of AC2 has facilitated screening for NG using non-invasive sampling in the first instance. Although culture has been advocated as the method of first choice within the United Kingdom[8] and it achieves 100% specificity, it is considered insensitive for urine specimens or vaginal swabs.[9] In our study, although we found supportive evidence for a gonorrhoea diagnosis for 37/38 women, only 31 of these were found on culture of an equivalent site. Three were found on culture of an additional site, and the remaining three women were partners of men with confirmed gonorrhoea. Without the benefit of this additional supportive evidence, only 31/38 would have been confirmed hence giving a much lower estimate of positive predictive value in this low prevalence population.

Other authors have suggested that routine confirmatory testing, even in populations with a low prevalence of gonorrhoea is not necessary following a positive result obtained using Gen-Probe Aptima Combo-2 testing.[10] However, as culture is the only practical method of determining an appropriate antibiotic susceptibility profile, it is essential to ensure that the standard of 95% of cases of genital gonorrhoea that are cured by first line antibiotic therapy is maintained.[11] Concerns regarding antimicrobial resistance resulted in the introduction of GRASP (The Gonococcal Resistance to Antimicrobials Surveillance Programme), which was created to monitor resistance. In 2004, 14.1% of GRASP isolates showed resistance to ciprofloxacin, compared with ceftriaxone which showed no resistance.[12] The British Association for Sexual Health and HIV therefore suggest third generation cephalosporins (ceftriaxone and cefixime) as first line therapy.[8]

AC2 testing may offer a more acceptable alternative to microscopy and culture of gonorrhoea. Further monitoring involving increased numbers of the screening population will be undertaken to ensure that the predicted efficacy of AC2 testing is a reliable indicator of the presence of gonorrhoea infection.

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CONTRIBUTORS

SL is the lead for the Liverpool and South Sefton Chlamydia Screening Programme who was responsible for monitoring the outcome of screening tests, and was also the co-coordinator of the writing of this paper; KJ is a health adviser who is employed within the screening programme and GUM, she has collected all of the follow up data from GUM, was part of the writing team, and has provided significant support in the management of screening clients; HM is the consultant clinical scientist at the laboratory where all testing was carried out, he has provided information and advice regarding testing platforms and positive predictive values, and has also directly contributed to the writing of this paper; AW is the chair of the local Chlamydia Screening Programme Steering Group, and has provided a significant contribution to the writing of this paper.

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